



## EVALUATION OF THE SERUM LEVEL OF ANTIOXIDANTS IN PATIENTS WITH BRAIN TUMOR

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### ABSTRACT

Intracranial neoplasm is one of the major health problems in both industrialized and developing countries. As in other chronic diseases, several factors, including reactive oxygen species, are involved in its development. The role of antioxidants in the development of brain tumors has been the subject of researches for many years. Their main effects are on pro-oxidants produced by normal cell metabolism processes. Vitamin C, ferritin, and ceruloplasmin are considered the most important antioxidants in the body.

**Material and Methods:** serum level of Vitamin C, ferritin, and ceruloplasmin of 102 people (patients = 51; controls = 51) were measured, using enzyme-linked immunosorbent assay (ELISA), and compared afterwards.

**Results:** Serum ferritin level was significantly associated with the risk of intracranial neoplasm. The same applies to the serum level of vitamin C, although to a lesser extent. On the other hand, there was no significant relationship between serum ceruloplasmin level and brain tumor.

**Conclusions:** Various studies have reported different results with regard to the relationship between antioxidants and intracranial neoplasm. The impact of these materials on refining of pro-oxidants in different body tissues could be an appropriate ground for reducing the risk of chronic diseases including tumors.

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### Introduction

The incidence of primary malignant brain tumors and other tumors of the central nervous system in 2012, after adjustment with demographic standards and age conditions, was 3.4 per 100,000 people. This amount in men and women was respectively 3.9 and 3.4 in that population, meaning that about 139,608 men and 116,605 women were diagnosed with primary tumors of the brain worldwide in that year (totally 256213 people). The incidence was greater in developed countries (5.1 per hundred thousand) than in developing countries (3.0 per hundred thousand) [1]. Primary brain tumors refer to tumors that develop inside the skull. Different species and subspecies of primary brain tumors have been identified, some of which are benign and some are malignant. Glioblastoma multiforme (GBM) and meningioma are the most common types of intracranial neoplasm [2]. Like other chronic diseases, several agents are involved in causing brain tumors. These include genetics, environmental factors, chemical agents, radiation, as well as reactive oxygen species (ROS) [6]. ROS are produced by living organisms and normal cell metabolism. They take part in physiological cellular processes with low and medium values; yet at high concentrations, they could have devastating effects on cellular components such as lipids, proteins, and DNA [7]. For a long

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time, food products containing antioxidants have been considered to prevent- if not cure – cancers [15,26,27]. Some studies have reported lower levels of serum and tumorous tissues antioxidants among patients with intracranial neoplasm compared with normal control group [14,15]. However, recent studies provide evidence to the contrary [9]. The role of antioxidants in the development of brain tumors has attracted the attention of a host of researchers, given that one could improve their levels through making changes to diets and lifestyles [12,16]. In case their relationship is confirmed, it will be possible to influence the incidence of intracranial neoplasm using various methods; moreover, they could be employed as a marker for following up and identifying risky groups as well as assessing the risk of brain cancers developing [24].

### Material and Methods

This research is a case-control study examining 51 patients diagnosed with meningioma and glioblastoma multiforme (GBM) who were hospitalized for surgery in the Neurosurgery Ward of Imam Reza Hospital (Tabriz) during 2016-2017. The control group, including 51 people matched in terms of sex and age, had referred to the hospital's Emergency due to minor injuries, showing mild symptoms such as nausea, they underwent CT scan but the results were normal, and thus they were discharged.

After they gave their written consent, patients' blood sample were taken a night before surgery in order to be sent for the routine tests. Then, their serums were separated and stored at  $-70^{\circ}\text{C}$ . Patients with intracranial neoplasm underwent surgery and their tumor samples were sent in for pathological examination. Patients who had tumors other than meningioma and GBM were dismissed from the study. In both case and control groups, patients with chronic diseases, diabetes, malnutrition, alcohol, and drug abuse were excluded. A questionnaire was filled out containing general information about age, sex, education, job, and smoking. After data and sample collection, the frozen serums were set to room temperature ( $20$  to  $27^{\circ}\text{C}$ ), so that the related antioxidants might be measured.

### Measuring Ferritin

Ferritin was measured by Monobind Inc kits and using enzyme-linked immunosorbent assay (ELISA). In this method, ferritin calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for ferritin) is added and the reactants mixed. Reaction results between the biotinylated ferritin antibody and native ferritin to form an immune complex that is deposited on the streptavidin coated well. The excess serum proteins are washed away via a wash step. Another ferritin specific antibody, labeled with an enzyme, is added to the wells. The enzyme labeled antibody binds to the ferritin already immobilized on the well. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the ferritin in the sample.

### Measuring Vitamin C

Vitamin C was measured by Vitamin C Assay, ZellBio, and GmbH kits using ELISA method. The 2,4-dinitrophenylhydrazin colorimetric method widely used for determination of ascorbic acid level in biological fluids. The procedure depends on the principle of oxidation of L-ascorbic acid to dehydroascorbic acid and 2,3-diketo-gulonic acid, followed by reaction with 2,4-dinitrophenylhydrazin. After treatment with sulfuric acid, a colored product is formed which is absorbed at 520 nm. Serum samples were left at room temperature for 5 to 10 minutes in order to clot. Then, they were centrifuged for 20 minutes (at 2000 to 3000 RPM) and collected carefully. Afterwards, 200  $\mu\text{l}$  of Tous Kit microplate solution was added and wells absorbance was read at 520 nm by ELISA reader/microplate reader.

### Measuring Ceruloplasmin

Ceruloplasmin was measured with nephelometric methods and with MININEPH HUMAN CAERULOPIASMIN kit. The determination of soluble antigen concentration by nephelometric methods involves a reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is scattered and detected by a photodione. The amount of light scattered is directly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Data were analyzed using STATA version 14. General characteristics of the investigated groups were presented in the form of a descriptive table representing numbers and percentage. Mean and standard deviation of antioxidants (ferritin...) in the two groups were obtained and analyzed using independent t-tests. Significance level was considered 0.05.

### Results

A total of 102 individuals were included in this study (51 cases and 51 controls). The mean age of cases was  $49.8 \pm 11.2$  years and controls was  $48.0 \pm 11.0$  (NS). 61% of both groups were male. 56.9% of cases were illiterate or have primary education while it was 35.35 among controls ( $p=0.03$ ). 47% of cases and 43% of control group were overweight or obese. Smoking was present in 18 % of both groups. About 22% of cases were from rural areas it was only 8% among control group ( $p=0.05$ ). (Table1)

**Table 1.** The Characteristics of study participants

General variables	Case N (%)	Control N (%)
<b>Age</b> Mean± SD	49.8±11.2	48.0±11.0
<b>Gender</b>		
Male	31 (61.8)	31(61.8)
Female	20 (39.2)	20 (39.2)
<b>Education</b>		
Illiterate/primary	29 (56.9)	18(35.3)
Literate	22	33
<b>Smoking</b>		
Yes	9 (18)	9(18)
No	41	41
<b>BMI</b>		
Normal	29 (56.9)	27 (52.9)
Overweight/obese	22 (47.1)	24 (43.1)
<b>Residency</b>		
Urban	40 (78.4)	47 (92.2)
Rural	11 (21.6)	4(7.8)

The mean concentration of ferritin was lower among cases than controls and it was the lowest among those cases with glioma compared to the control group. There is a statistically significant difference in the mean level of Vitamin C between cases and controls ( $p=0.02$ ). The mean level of Vitamin C was lowest among those cases with meningioma. The mean level of seroplasmin was lower among cases than controls but it was not statistically significant. No obvious difference was observed by type of tumor. (Table 2)

**Table2.** The Concentration of some antioxidants in stud groups

	Cases (n=51)	Control (n=51)	Glioma(n=16)	Menengioma (n=35)
Ferritin	94.96±128.91	143.95±165.71	73.84±52.90	104.62±151.32
Vit C	2.03±1.40	2.62±1.22	2.21±0.95	1.94±1.57
Seroplasmin	0.35±0.12	0.38± 0.18	0.34±0.09	0.36±0.13

In the next step, serum antioxidants were modeled as quartiles based on distribution among control group. Odds Ratios (ORs) of brain tumor according to quartiles of serum antioxidants were calculated and p for trend was provided (Table 3).

**Table 3.** The ORs of brain tumor according to antioxidant quartiles

	Q1	Q2	Q3	Q4	P for trend
Ferritin					
OR	Ref (1)	0.43	0.30	0.24	0.01
95%CI	-	0.11-1.61	0.06-1.42	0.07-0.77	
Vitamin C	Ref (1)				

OR	-	0.34	0.46	0.35	0.06
95%CI	-	0.11-1.10	0.16-1.31	0.12-1.02	
Seroplasmin	Ref (1)				
OR	-	0.27	0.59	0.96	0.81
95%CI	-	0.08-0.98	0.19-1.80	0.35-2.63	

Serum ferritin was significantly associated with brain tumor risk, as the level of ferritin increased the risk of brain tumor decreased. However it was barely evident about the serum Vitamin C.

Although the OR in the highest quartile of seroplasmin was higher than that in lowest quartile. There was no significant association between the brain tumor and seroplasmin level.

After adjustment for other variable (age, sex, education, residency and BMI) the OR of brain tumor was the lowest in the highest quartile of ferritin level (OR=0.25 , 95% CI=0.07-0.89). The same was true for the VitaminC (OR=0.31 95% CI=0.09-0.97).

### Discussion

The results of this study indicated that, among the investigated antioxidants, ferritin level in patients with intracranial neoplasm - especially people with gliomas - is low. pang et al. (2015) studied the role of ferritin in the biology of cancers. Their study indicated that ferritin level in tumoral cells is high [25]. Al Khateeb et al. (2013) showed, in a review study, that ferritin level is high in tumoral tissues and the serum of patients with different types of tumor including glioblastoma [24]. They suggested high levels of serum ferritin as a risk factor, independent from consumption of oral iron, for developing different types of cancers. These results are incompatible with those of our study. Given the recognized role of ferritin antioxidant and regarding the fact that both of the aforementioned studies reported high levels of ferritin in tumoral tissues, their reported results are in contrast to ferritin's known physiological effects. In our study, vitamin C had similar results, especially in patients with meningioma. In a study conducted by Hamad et al. (2015), the level of several vitamins including Vitamin C was measured in the saliva, serum, cerebrospinal fluid, and tumoral tissue of patients with intracranial neoplasm, and compared with that of normal people; the result was that there were low levels of the vitamins in question among patients [28]. Also, Du et al. (2012) reported that plasma concentration of ascorbic acid has an inverse relationship with the risk of developing different cancers [15], which is in line with the results of the current study. In a meta-analysis by Zhou et al.(2015), it is noted that oral consumption of vitamin C might reduce the risk of developing gliomas [29]. Similarly, Kyritsis et al. (2012) mentioned that ascorbyl stearate, one of the lipophilic derivative of vitamin C, has anti-proliferative and apoptotic effects on glial cells [16], which reaffirms our results. In another study, Michels et al. (2015) investigated those genetic variations that are effective on the homeostasis of vitamin C, and it was observed that some of those genes could increase the absorption of vitamin C as well as its amount in serum. This finding may justify the difference in the level of vitamin C of patients studied in the present study [30].

In our study, there was not a significant difference in the level of ceruloplasmin between patients of the case and control groups. Wu et al. (2000) compared the level of antioxidants in patients with intracranial neoplasm with the control group and reported that this level was lower in most of patients than in the control group; however, they mentioned that the level of ceruloplasmin in both of studied groups did not have a significant difference[6], which is consistent with the current study.

In conclusion, given that it is possible to a great extent to analyze and control antioxidants, it seems that their prophylactic function and, thus, their application in treatment and screening have become the subject of numerous recent studies. Studying antioxidants at a greater volume as well as in molecular and cellular levels, one can discover their main role in the formation and growth of tumors (including brain tumors) and resolve the many contradictions existing in the literature. As well, by doing studies on the impact of nutrition, genetics, and lifestyle on effecting differences in serum levels among diverse people, one might introduce solutions into the general policies of public health for controlling and preventing cancer.

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